Biosurfactant of a bacterial system reverses the coffee ring effect

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The deposition of material at the edge of evaporating droplets, known as the ”coffee ring effect”, is caused by a radially outward capillary flow. This phenomenon is common to a wide array of systems including colloidal and bacterial systems. The role of surfactants in counteracting these coffee ring depositions is related to the occurrence of local vortices known as Marangoni eddies. We have shown that these swirling flows are universal, and not only lead to a uniform deposition of colloids but also occur in living bacterial systems. Experiments on *Pseudomonas aeruginosa* suggest that the auto-production of biosurfactants plays an essential role in creating a homogeneous deposition of the bacteria upon drying. Moreover, at biologically relevant conditions, intricate time dependent flows are observed in addition to the vortex regime, which are also effective in reversing the coffee ring effect at even lower surfactant concentrations.

When the contact line of an evaporating droplet on a solid substrate is pinned, the surface tension sets up a capillary flow directed towards the edges. This capillary flow carries suspended matter to the edges of the droplet and results in deposition near the contact line. This process is commonly referred to as the ”coffee ring effect”[1]. However, there are several factors which can counteract this flow within the droplet, all of which derive from the occurrence of gradients in surface tension, known as Marangoni effects[2]. As Hu and Larson have shown, small quantities of contaminants result in significant Marangoni stresses that suppress these temperature-induced vortices[3].

Surfactants can also impact significantly on the coffee ring effect as they have a dramatic effect on the surface tension gradients within a droplet. For insoluble surfactant systems, Stebe and coworkers demonstrated the occurrence of Marangoni-Benard cells with an intricate pattern formation, due to the coupling between local temperature gradients and thermodynamic transitions at the surface[4]. For soluble surfactants on the other hand, recent work qualitatively investigated the effect of sodium dodecyl sulfate (SDS) on the evaporation of aqueous drops. The compositional Marangoni stresses within these droplets created localized vortices, termed ”Marangoni eddies”. These eddies have a dramatic effect on particulate deposition, giving a rather uniform deposition of material during evaporation[3].

Although this has received little or no attention so far, the coffee ring effect can also be observed in bacterial systems. This leads to very dense deposits of bacteria near the contact line. However when we performed simple experiments of evaporating droplets containing live bacteria (*Pseudomonas aeruginosa*), no coffee ring depositions were observed, as seen in figure 1(b). In contrast in figure 1(a), a heterogeneous deposition of the bacteria, concentrated at the contact line, is obtained using a knock-out mutant (where the gene responsible for the production of biosurfactant was switched off). Our observations suggest that the mutant bacteria were subject to the capillary flow whereas the wild type bacteria gave a nearly uniform distribution of bacteria after drying due to the production of the biosurfactant rhamnolipid, which counteracts the capillary flow.

To further investigate the role of surfactants on biological systems, while maintaining quantitative control of the surfactant concentration and properties, we performed a study on the role of exogeneously supplied non-ionic surfactants on the dynamics of eGFP-expressing *Escherichia coli* systems during drying. *P. aeruginosa* is not an ideal system to investigate these dynamics using confocal microscopy in detail: due to its pathogenic nature and due to the high bacterial density under swarming conditions, which leads to a difficulty in imaging. Additionally, to have more control over the surfactant type and its concentration in the droplet, non-biosurfactant producing *Escherichia coli* was used to enable the systematic (more quantitative) study on the influence of exogeneously added surfactant. Our experimental observations are compared to numerical calculations to rationalize our

observations.

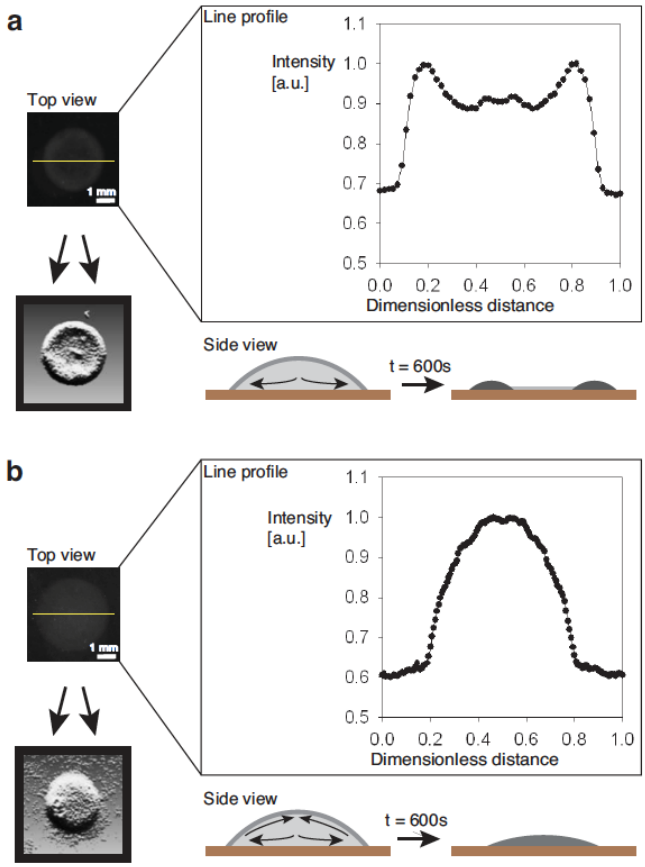

Figure 1 *Pseudomonas aeruginosa* produces a biosurfactant that induces a Marangoni flow and inhibits the coffee ring effect. Droplets of liquid culture of *Pseudomonas aeruginosa* are spotted on solid agaragar plates for a rhamnolipid production deficient mutant (a) and a wild type (b). The mutant undergoes the capillary flow producing a coffee ring during drying, leading to bacteria being heaped up at the contact line. The wild type gives a more uniform distribution of cells after drying due to the production of the biosurfactant rhamnolipid, which induces a Marangoni flow and inhibits the coffee ring effect. Intensity line profiles of the respective colonies are shown to indicate this phenomenon and statistical analysis confirms these findings. For both, a post-processed image of an example of a colony is shown, as a guide of the eye. The scale bars represent 1 mm.

These bacterial (and other, colloidal systems) were studied to determine the role of surfactants in more detail and to explore the link with the different observed evaporation patterns. Different types of exogeneously supplied surfactants were used, including rhamnolipids, in order to investigate the effect of the surfactant (Triton X-100, Tween 80, rhamnolipids and Triton X-114) were tested in different concentrations.

Interestingly, we saw that the auto-production or exogenous addition of a soluble non-ionic (bio)surfactant induces complex flow patterns in a region near the edge of an evaporating droplet. This is due to the generation of a Marangoni flow, itself being created by a heterogeneous distribution of surfactant molecules along the interface by an outward capillary flow due to evaporation. The Marangoni stresses generated lead to full-blown vortices **at high concentrations** or after long drying times, but for **lower concentrations**, an oscillatory motion can be observed in the regime where capillary and Marangoni flow are equal in strength. In all cases upon addition of surfactants, **it is the underlying physical phenomenon of the interplay between the capillary flow and the Marangoni stresses that leads to the observed macroscopic deposition patterns.** Our observations show that even far above the critical micelle concentration, an increase in the addition of surfactant still has a remarkable influence, in contrast to routine surface tension measurements. Our experiments indicate that biosurfactants play a significant role in the distribution of bacterial systems during drying, in general, in addition to the recently discovered role of self-produced surfactants in transport and movement processes. In conclusion, we prove that Marangoni stresses can generate sufficiently strong forces to drive both surface and bulk flows, either in swarming colonies or during drying of bacterial systems[6].

References

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